NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

OLEIC ACID DIETHANOLAMINE CONDENSATE

(CAS NO. 93-83-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

July 1999

NTP TR 481

NIH Publication No. 99-3971

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical s carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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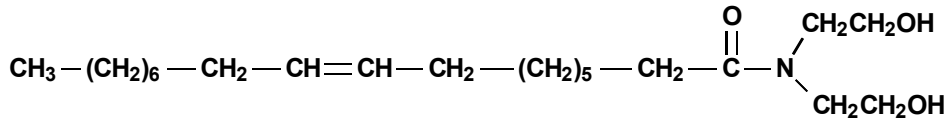
ABSTRACT



OLEIC ACID DIETHANOLAMINE CONDENSATE

CAS No. 93-83-4

Chemical Formula: $C_{22}H_{43}NO_3$ Molecular Weight: 387.68

Synonyms: Diethanolamine oleate; diethanolammonium oleate; (Z)-9-octadecenoic acid, compound with 2,2′-imnobis(ethanol) (1:1); oleamide diethanolamine

Oleic acid diethanolamine condensate is widely used as an emollient, thickener, and foam stabilizer present in cosmetic formulations of bath additives, shampoos, conditioners, lipsticks, and hair dyes. Male and female F344/N rats and B6C3F$_1$ mice received dermal applications of diethanolamine in 95% ethanol for 13 weeks or 2 years. Genetic toxicology studies were performed in *Salmonella typhimurium* and L5178Y mouse lymphoma cells.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were administered 0, 25, 50, 100, 200, or 400 mg oleic acid diethanolamine condensate/kg body weight in ethanol dermally for 13 weeks. All male and female rats survived until the end of the study. The final mean body weights and body weight gains of 200 and 400 mg/kg males and the mean body weight gain of 400 mg/kg females were significantly less than those of the vehicle controls. The only chemical-related clinical finding was irritation of the skin at the site of application in most males administered 100 mg/kg or greater and in all females administered 50 mg/kg or greater. Segmented neutrophil counts were increased relative to the vehicle controls in the 400 mg/kg male group on days 5 and 19, in the 200 mg/kg female group on day 19 and at week 13, and in the 400 mg/kg female group on days 5 and 19 and at week 13. Alkaline phosphatase concentrations were significantly increased in the 200 mg/kg male group on day 19, the 200 mg/kg female group at week 13, and in the 400 mg/kg groups of males and females at week 13. Kidney weights of 200 and 400 mg/kg females were significantly greater than those of the vehicle controls. Lesions of the skin at the site of application included epidermal hyperplasia, parakeratosis, chronic active dermal inflammation, suppurative epidermal inflammation, and sebaceous gland hypertrophy in dosed rats. The severities of these lesions generally increased with increasing dose.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice were administered 0, 50, 100, 200, 400, or 800 mg oleic acid diethanolamine condensate/kg body weight in ethanol dermally for 13 weeks. All male and female mice except one 800 mg/kg male survived until the end of the study. Final mean body weights and body weight gains of 800 mg/kg males and females and 400 mg/kg females were significantly less than those of the vehicle controls. Clinical findings in dosed mice included irritation of the skin at the site of application. Irritation occurred in all surviving dosed males and in most females administered 100 mg/kg or greater and progressed to ulcer in one 800 mg/kg male. The heart weights of 400 and 800 mg/kg males and females and

200 mg/kg females and the kidney weights of 50, 100, and 400 mg/kg males were significantly greater than those of the vehicle controls. Relative to the vehicle controls, the liver weights were increased in all dosed groups. Lesions of the skin at the site of application in dosed mice included epidermal hyperplasia, parakeratosis, suppurative epidermal inflammation, chronic active dermal inflammation, sebaceous gland hypertrophy, and ulcer. The severities of these lesions generally increased with increasing dose.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were administered 0, 50, or 100 mg oleic acid diethanolamine condensate/kg body weight in ethanol dermally for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of dosed male and female rats was similar to that of the vehicle control groups. Mean body weights of 100 mg/kg males were slightly less than those of the vehicle controls throughout most of the study. Mean body weights of 100 mg/kg females were less than those of the vehicle controls beginning at week 24. The only significant treatment-related clinical finding was mild to moderate irritation of the skin at the site of application in dosed males and females.

Pathology Findings

The predominant effects of oleic acid diethanolamine condensate administration were minimal to moderate nonneoplastic lesions of the skin at the site of application in dosed rats. These lesions included epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis, parakeratosis, chronic active dermal inflammation, and ulcer.

2-YEAR STUDY IN MICE

Groups of 55 male and 55 female mice were administered 0, 15, or 30 mg oleic acid diethanolamine condensate/kg body weight in ethanol dermally for 2 years. Five animals from each group were evaluated at 3 months for gross lesions and skin histopathology.

Survival, Body Weights, and Clinical Findings

Survival of dosed male and female mice was similar to that of the vehicle control groups. Mean body weights of dosed males and of 15 mg/kg females were similar to those of the vehicle controls throughout the study. Mean body weights of 30 mg/kg females were less than those of the vehicle controls from week 76 until the end of the study. The only significant treatment-related clinical finding was irritation of the skin at the site of application in 30 mg/kg males.

Pathology Findings

The incidences of epidermal hyperplasia, sebaceous gland hyperplasia, and chronic active inflammation of the dermis in all dosed groups were significantly increased relative to the vehicle controls at 3 months and at 2 years. The increased incidences of hyperkeratosis in dosed males at 3 months and in dosed males and females at 2 years, of parakeratosis in 30 mg/kg males at 3 months and 2 years, and of ulcer in 30 mg/kg males and exudate in 30 mg/kg males and females at 2 years were also attributed to chemical administration.

GENETIC TOXICOLOGY

Oleic acid diethanolamine condensate was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535, with or without S9 metabolic activation enzymes. In addition, it did not induce mutations in mouse L5178Y lymphoma cells treated with or without S9.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of oleic acid diethanolamine condensate in male or female F344/N rats administered 50 or 100 mg/kg or in male or female $B6C3F_1$ mice administered 15 or 30 mg/kg.

Dermal administration of oleic acid diethanolamine condensate to male and female rats was associated with epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis, parakeratosis, chronic active inflammation of the dermis, and ulceration of the skin

at the site of application. Dermal administration of oleic acid diethanolamine condensate to mice was associated with epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis, chronic active inflammation of the dermis, and exudate of the skin at the site of application in males and females and parakeratosis and ulcer of the skin at the site of application in males.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Oleic Acid Diethanolamine Condensate

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses in ethanol by dermal application	0, 50, or 100 mg/kg	0, 50, or 100 mg/kg	0, 15, or 30 mg/kg	0, 15, or 30 mg/kg
Body weights	100 mg/kg group slightly less than vehicle control group	100 mg/kg group less than vehicle control group	Dosed groups similar to vehicle control group	30 mg/kg group less than vehicle control group
Survival rates	8/50, 10/50, 14/50	15/50, 18/50, 14/50	41/49, 35/50, 34/50	34/50, 30/50, 35/50
Nonneoplastic effects	Skin (site of application): epidermal hyperplasia (0/50, 49/50, 47/50); sebaceous gland, hyperplasia (1/50, 45/50, 45/50); hyperkeratosis (0/50, 44/50, 40/50); parakeratosis (0/50, 10/50, 11/50); chronic active dermal inflammation (0/50, 48/50, 41/50); ulcer (0/50, 7/50, 6/50)	Skin (site of application): epidermal hyperplasia (3/50, 50/50, 50/50); sebaceous gland, hyperplasia (2/50, 48/50, 49/50); hyperkeratosis (1/50, 38/50, 31/50); parakeratosis (2/50, 27/50, 43/50); chronic active dermal inflammation (2/50, 44/50, 48/50); ulcer (3/50, 20/50, 36/50)	Skin (site of application): epidermal hyperplasia (1/49, 40/50, 47/50); sebaceous gland hyperplasia (1/49, 21/50, 34/50); hyperkeratosis (1/49, 38/50, 37/50); parakeratosis (0/49, 2/50, 8/50); chronic active dermal inflammation (0/49, 34/50, 50/50); ulcer (0/49, 0/50, 7/50); exudate (1/49, 3/50, 9/50)	Skin (site of application): epidermal hyperplasia (0/50, 43/50, 50/50); sebaceous gland hyperplasia (0/50, 39/50, 46/50); hyperkeratosis (0/50, 36/50, 42/50); chronic active dermal inflammation (0/50, 40/50, 49/50); exudate (0/50, 0/50, 6/50)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative with and without S9 in strains TA97, TA98, TA100, and TA1535			
Mouse lymphoma gene mutations:	Negative with and without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on oleic acid diethanolamine condensate on 9 December 1997 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 9 December 1997 the draft Technical Report on the toxicology and carcinogenesis studies of oleic acid diethanolamine condensate received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of oleic acid diethanolamine condensate by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* in male or female F344/N rats or male or female B6C3F$_1$ mice.

Dr. Goldsworthy, a principal reviewer, agreed in principle with the proposed conclusions. He asked whether equivocal evidence was considered for the occurrence of interstitial cell adenoma of the testis in male rats. He noted that this response appeared to be increased with respect to the most suitable controls, the concurrent controls and those from the three other diethanolamine studies. Dr. J.K. Haseman, NIEHS, responded that one of the two dermal studies in the historical database had a control rate for testicular neoplasms in rats that was higher than the rate in the 100 mg/kg group in this study. Also, no increases in the incidences of these neoplasms were seen in the three other diethanolamine studies.

Dr. I. Russo, the second principal reviewer, agreed with the proposed conclusions. She wondered if the neoplastic responses in this study would have been similar to those in the two other diethanolamine condensate studies if the free diethanolamine content had been similar rather than lower. She suggested the addition of a graph showing the diethanolamine content of each condensate (Figure 5, p. 48).

Dr. Carlson and others expressed concern about the large number of impurities in the test material. Dr. C.S. Smith, NIEHS, noted that the results of the purity analyses were in the appendix and that the impurities were mainly other fatty acids, free diethanolamines, or unidentifiable organic impurities. Dr. J.R. Bucher, NIEHS, said that the NTP would determine if there is a purity grade material designation for these diethanolamides and, if so, that information would be added to the title of each Technical Report.

Dr. Goldsworthy moved that the Technical Report on oleic acid diethanolamine condensate be accepted with the revisions discussed and the conclusions as written for male and female mice, *no evidence of carcinogenic activity*. Dr. I. Russo seconded the motion, which was accepted by seven yes votes and one abstention (Dr. Bus).